

mutant of hGH bound the GH receptor, although more weakly than does wild-type GH ( $K_D$  0.53 nM vs. 0.34 for wild-type). They did not determine its action in vivo, and hence did not know whether it has GH antagonist activity (both agonists and antagonists would bind the GH receptor; the difference is observable only in vivo, e.g., by observing growth rates). Indeed, it is still not known whether it would have such activity, although applicants would expect it to.

In Serial No. 08/313,505 on October 21, 1997, the PTO ruled that since "it was known in the prior art that prolactin and growth hormone agonize each other", and "one of ordinary skill" would have expected the "hPRL (111-129)" mutant to have "agonistic activity", in the absence of evidence that the mutant in fact had antagonistic activity, the Examiner could not reject under §102. The PTO further held that even if applicants' specification would support a prediction of activity, that could not be used against applicants.

Nonetheless, the possibility remains that at some future date, it could be determined that Cunningham's "hPRL (111-129)" mutant was a GH receptor antagonist, and hence that his expression vector was a DNA molecule within the claims previously presented.

Hence, as a precaution, the DNA molecule claims have been amended to excise this specific mutant.

Obviousness is not at issue. First, there was no suspicion that a GH could be mutated at the residue corresponding to bGH-119 (e.g., gHG-120) to convert the protein into a GH antagonist. Second, the "hPRL (111-129)" mutant exhibited reduced affinity, so it was a "dead end" from the standpoint of anyone seeking to improve on GH's agonist activity. Hence, there is no need to exclude any mutants other than the exact one made by Cunningham.

The proviso has not been placed in the method-of-use claims since Cunningham's DNA was not used, and would not have been expected to be useful, in imparting a GH antagonist activity to a subject. Hence these claims could not be anticipated or rendered obvious.

2. A new Abstract of the Disclosure is enclosed which emphasizes the use of DNA.

3. The description of the Figures has been corrected.

4. The incorporation by reference issue is moot in view of the issuance of 08/313,505 as USP 5,681,809. A copy of the '809 patent is enclosed.

5. The Examiner is thanked for observing that claims 10-28 and 34-44 are directed to an allowable product, and consequently rejoining method-of-use claims 46-61.

6. In response to the indefiniteness rejections set forth in points 12 and 24 of the office action, claims 29 and 46 have been amended to recite growth hormone receptor antagonist activity. Thus, the type of antagonist activity is now claimed.

7. With regard to the terminology "human or animal subject" in claim 46, (questioned in point 24 of the office action) the Examiner will appreciate that, while to a biologist, "humans" are "animals", the common meaning of "animals" excludes "humans". The phrase "human or animal subject" was employed to make it clear that the claim covered both humans, and nonhuman animals.

After further reflection, we agree that the reference to "humans" is unnecessary. The term "animal" is said at page 34, lines 34-35 to be "intended to include humans". The reference to "nonhuman...animal" in claims 45 and 61 also imply that without this limitation, "animal" (or "mammal") will read on humans.

Hence claim 46 has been amended to refer merely to a

"subject", which may be a human, or a nonhuman animal. Humans are of course, "mammalian subjects" within the meaning of the claim.

8. In response to point 25 of the office action, the words "the antagonist" in claims 47, 51 and 52 seemingly find antecedent basis in base claim 10 ("said polypeptide having growth hormone receptor antagonist activity"). However, applicants have amended claim 10 so it now refers to "encoding an antagonist which is a polypeptide...."

9. In point 26, the Examiner has questioned the meaning of "smaller-than-normal size" in claim 61. Admittedly, "size" could be quantified as body weight, body length, body girth, etc. However, the only "size" parameter specifically discussed in the application is "body weight" (see ordinate of Fig. 6). Hence, claim 61 has been amended to refer to "weight" rather than size.

10. The Examiner has rejected claims 29-33 for lack of enablement, while conceding that the other polypeptide claims are allowable. Both claim 29 and claim 10 require at least 50% amino acid sequence identity with a vertebrate growth hormone (a structural limitation), and that the polypeptide have a growth hormone receptor antagonist activity (a functional limitation influencing the underlying structure). They also both require the critical change at the amino acid position corresponding to bGH-119. The difference between them is that claim 10 specifically limits which amino acids may be changed, and to what, while claim 29 allows that to remain implicit in the functional limitation.

The Examiner contends that the additional limitations set forth in claim 10 "provide guidance" as to the genetic engineering of GH antagonists and are "necessary structural limitations which would provide the functional limitations which

are recited in the claims".

We believe that the examiner mistakes the proper role of claim limitations. It is not to provide "guidance" to the person skilled in the art; that is the purpose of the specification. The claims are intended to define the metes-and-bounds of the invention.

The additional structural limitations of claim 10 crystallize certain guidelines expressly set forth in the specification. However, the Examiner has not established that disregarding these guidelines would necessarily, or even frequently, result in loss of GH antagonist activity, at least in the case of a mutant which is still at least 50% identical with vertebrate growth hormone.

Suppose for example, that we were contemplating a nonconservative mutation at a position which is invariant in the vertebrate GH family, and where an alanine substitution resulted in a more than tenfold loss of affinity. There may be substitutions which, while not "conservative" as defined by the specification, are more conservative than alanine. For example, if the original residue was glutamic acid, one might replace it with lysine instead of alanine. This results in a reversal of charge, but both lysine and glutamic acid are much more hydrophilic than alanine. Within the vertebrate growth hormone family, as set forth by Watahiki, et al., J. Biol. Chem., 264:312 (1989) (copy enclosed) there are ten instances in which Glu in one GH is aligned with Lys in another (see spec., page 26, line 21 to page 28, line 17, and especially page 27, lines 4-5). Thus, the limitations of claim 10 do not exhaust all of the guidance which the specification gives the art.

The very fact that a change in a residue strongly impacts binding indicates that, while caution is called, the opportunity

exists to actually enhance binding. It is clear that hGH is not fully optimized for binding to the GH receptor, since Cunningham and Wells, Science, 244:1081 (1989) discovered several mutations which enhance binding. The present specification teaches that the effect of varying a residue may be systematically explored by random mutagenesis. See page 22, lines 6-10 page 23, line 4. Such exploration has since been carried out by others, see WO97/11178, Example II for hGH residues in libraries "mini-helix-1" (41, 42, 45, 46), "loop-A" (54, 56, 58, 64), "helix-1" (10, 14, 18, 21) and "helix-4b" (167, 171, 175, 179). WO 97/11178 goes on to show that two multiple substitution mutants of hGH, which combine applicants' G120R mutation with mutations suggested by alanine-scanning, homologue-scanning, or combinatorial mutagenesis studies, have GH antagonist activity (see Exs. XI-XIV). See also Fuh, et al., Science, 256:1667-9 (1992) for a related antagonist inspired by applicants' G120R mutant and Cunningham's Ala-scan data.

Another teaching of the specification which is not reflected in claim 10 appears at page 15, lines 1-18:

The three dimensional structure of porcine GH has been determined by X-ray diffraction and compared to that of other GHs. Abdel-Meguid et al., Proc. Natl. Acad. Sci. USA 84:6434 (1987). Like the other GHs thus studied, it is a single domain protein arranged as a four helix bundle with the helices in an antiparallel relationship. Its four helices are made up of residues 7-34, 75-87, 106-127 and 152-183. For X-ray studies of bGH and hGH, see Bell et al., J. Biol. Chem. 260:8520-25 (1985) and DeVos et

al., Science 255:306-312 (1992). The three-dimensional structures of other GHs may be deduced by comparison of the sequences with due regard for the secondary structure tendencies of substituted amino acids. Detailed structural models of GH may, in conjunction with the information provided in this application, be used by the person of ordinary skill in the art to introduce one or more mutations into GH antagonists without interfering with ability of a given GH antagonist to serve as a GH antagonist.

The utility of the 3D structure is further explained at page 25, lines 5-16:

Abdel-Meguid et al. (1987) determined the three-dimensional structure of recombinant methionyl porcine GH, and suggested that it revealed the "general three-dimensional fold" of the GHs. The three-dimensional structure can be used to identify interior and surface residues; generally speaking, proteins mutated at surface residues (other than the receptor binding site) are more likely to remain functional. However, Creighton and Chothia, Nature 339:14 (1989) discuss the toleration of mutations at buried residues. The structure may also be used to determine flexible surface "loops"; proteins are more tolerant of deletions and insertions in such regions.

It has indeed been shown that by mutating human prolactin

(which has only 23-26% identity with human GH) at only eight positions with substitutions which individually were known to enhance the affinity of human GH for its receptor, one raises prolactin's affinity for the human GH receptor from 1/100,000th to 1/6th that of human GH. Cunningham, et al., Science, 247:1461-5 (1990) (copy enclosed).

Claims which define a polypeptide or a DNA encoding a polypeptide, primarily by its percentage identity with a reference polypeptide sequence and, in some cases, the biological activity of the polypeptide, have frequently been granted by the PTO, reflecting a consensus that the combination of a sequence identity limitation and a functional limitation is sufficient to satisfy 112/1.

<u>Patent</u>	<u>%</u>	<u>Reference Polypeptide</u>
Bell 4,761,371 claim 8	40%	insulin receptor
Hoffman 5,545,727 claim 9	75%	human alpha globin
Bouma 5,656,484 claim 1 (DNA encoding protein)	95%	wild type mature human protein S
Walsh 5,646,026 claim 1 (DNA encoding protein)	70%	residues 162-186 of pro-Ribosome inactivating protein
Colman 5,663,294 claim 1	50%	residues 124-132 of mature human kininogen heavy chain

USSN - 08/488,164

Tripp 5,681,724 claim 1	55%	one of four parasitic helminth MIF proteins
Margolis 5,648,465 claim 2	80%	rat neurocan (but limited to naturally occurring mammalian neurocans)
Stafford, USP 5,268,275 claim 13	75%	vitamin K dependent carboxylase
USP 5,304,640 claim 2	40%	selectin ligand protein
USP 5,670,335	70%	mammalian inward-rectifying potassium channel protein IRK1
Fair, USP 5,187,155	95%	anticoagulant peptide
USP 5,538,892	70%	transforming growth factor-beta type I receptor MISR4
Deeley, USP 5,489,519	70%	multidrug resistance-associated protein MRP

Moreover, USP 5,646,014 contains a claim drawn to a peptide "homologous" to a reference polypeptide which "exhibits an antibacterial activity".

In Ex parte Mark, 12 USPQ2d 1904 (BPAI 1989), the Board was presented with a claim to:

1. A synthetic mutein of a biologically active native protein in which native

protein has at least one cysteine residue that is free to form a disulfide link and is nonessential to said biological activity, said mutein having at least one of said cysteine residues substituted by another amino acid and said mutein exhibiting the biological activity of said native protein.

This claim was not limited to mutants of a particular protein, like hGH, but rather included all proteins with cysteine residues. The Examiner was of the opinion that merely sequencing all of the possible proteins would require undue experimentation.

The Examiner conceded that successful embodiments were shown, but characterized them as "limited", and, in view of the "established unpredictability" of the effect of site-specific mutagenesis, expressed concern as to the number of residue positions and proteins which would have to be tested to obtain even one alternatively biologically active mutein.

There was no doubt that some cysteine residues would indeed prove to be essential. In CSF-1, all seven possible Cys→Ser mutations were known to substantially reduce activity.

The Board nonetheless reversed the rejection. Given that three proteins (1FN-beta, IL-2 and TNF) had been shown to have non-essential cysteine residues which could be replaced without loss of biological activity, and that the claims "all require that the mutein produced retain the biological protein of the native protein" (thus excluding the CSF-1 counterexample). The Board held that for a given protein having cysteine residues, it would not require undue experimentation to determine which, if any, of its cysteine residues could safely be replaced.

While the Mark claim is narrower than the present claim in the sense that it limits mutation to replacement of cysteine

residues, it is broader in that the reference protein is unlimited (other than that it be naturally occurring and biologically active). The Mark opinion makes it clear that an activity limitation can be used to exclude what would otherwise be an inoperative embodiment and thereby save a claim to subject matter with "established unpredictability".

11. As a potential alternative or supplement to the challenged claim 29, we have presented new claim 64, which like claim 10, specifically limits the nature and site of the mutations, but more broadly defines the reference hormone. Claim 64 requires that the encoded polypeptide be at least 50% identical with the amino acid sequence of "a first reference vertebrate hormone selected from the group consisting of vertebrate growth hormones, prolactins and placental lactogens".

The growth hormones, the prolactins, and the placental lactogens are recognized as being members of a family of homologous proteins. See Russell, et al., J. Biol. Chem., 256:296-300 (1981), discussed at spec., page 16, lines 29-34; Watahiki, et al., J. Biol. Chem., 264:312 (1989), discussed at spec., page 15, line 35, to page 16, line 28. Indeed, the specification expressly refers to human placental lactogen and prolactin as "homologous hormones", see page 16, lines 34-36.

While the wild-type prolactins and the placental lactogens do not have the growth-promoting activities of the wild-type growth hormones, it has been shown that some growth hormone/placental lactogen chimeras, and some growth hormone/prolactin chimeras, can bind the hGH receptor. See Cunningham, et al., Science, 243:1330 (1989) (copy enclosed), discussed at page 16, line 34 to page 17, line 7 and especially Cunningham Table 1 hGH mutants hPRL (22-33), hPRL (88-95), hPRL (97-104), hPL (88-95), hPRL (97-104), hPL (109-112), hPRL (111-

129), and hPRL (126-136). Moreover, it has been shown that by introducing merely eight mutations into human prolactin, its binding affinity for the hGH receptor may be increased over 10,000-fold. Cunningham, et al., Science, 247:1461-5 (1990) (copy enclosed).

In the case of human placental lactogen, which is 85% identical in sequence to hGH, but has 2300-fold weaker affinity for the hGH receptor (despite a similar affinity for the prolactin receptor), the affinity of hPL for the hGH receptor can be increased over 1400 fold (so it is only 1.6-fold weaker than hGH), by simultaneously introducing five mutations. Lowman, et al., J. Biol. Chem., \_\_\_\_:10982-8 (1991) (copy enclosed). Four of these (V4I, D56E, M64R, M179I) were inspired by the aligned hGH sequence (see Lowman Fig. 1), and the last (E174A) is one found by alanine-scanning mutagenesis of hGH to cause a 4.5-fold improvement in binding affinity for the hGH receptor. (The five mutations incidently reduced the affinity for the prolactin receptor 1000-fold.)

While the affinity-increasing and receptor-antagonizing mutations of hPL and hPRL have not yet been combined, in view of the general additivity of mutations observed, it is reasonably expected that such a combination would result in a higher affinity antagonist, especially in view of the successful combination described in Examples III, IV and XIII of Genentech, WO97/11178, copy enclosed.

The mutation at the residue corresponding to bGH-119 has been shown to convert human growth hormone and human placental lactogen, which normally agonize the prolactin receptor, into antagonists of the prolactin receptor. Fuh and Wells, J. Biol. Chem., 270:13133-7 (1995) (copy enclosed).

Hence, new claim 64 includes mutants of prolactins and

placental lactogens which have growth hormone antagonist activity.

New claim 64 also requires that the polypeptide antagonize an activity of a vertebrate growth hormone. This need not be a growth promoting activity; GHs can have other activities, such as lactogenic activity.

The specification recognizes that growth hormones have activities other than promoting growth, and hence that applicants' antagonists may be useful for antagonizing other GH-mediated activities. Thus, at page 20, lines 4-8, it states:

A GH mutein may be considered an antagonist, even if it lacks growth-inhibitory activity, if it antagonizes another GH-mediated activity, e.g., diabetogenic, glomerulosclerotic, hypercholesterolemic, tumorigenic or retinopathic activities.

At page 2, lines 4-6, it declares

Exogenous administration of bGH to cattle increases milk production, feed efficiency, growth rate, and the lean-to-fat ratio, and decreases fattening time.

Increasing milk production is a lactogenic activity. Lactogenic activity is not limited to bovine GH. See Ashkenazi, et al., Endocrinology, 121:414-419 (1987). Aviv, GB 2,073,245 (cp. USP 4,571,835) which is cited on page 2, lines 15-17 and is incorporated by reference, refers to the lactogenic activity of hGH, which is therefore one of the activities of GH which may be antagonized.

Hence, new claim 64 does not require GH receptor antagonist activity, since GH may act on prolactin and other receptors, too.

Claim 64 would read on GH mutants which antagonize the lactogenic<sup>2</sup> or diabetogenic<sup>3</sup> or adipogenic<sup>4</sup> activities of GHs, or act as anticholesterolemic agents<sup>5</sup>.

12. The Examiner has rejected claims 46-60 for alleged lack of enablement on grounds of (a) lack of an experimental showing that administration of the claimed DNA to humans leads to an alleviation of any specific diseases disease symptom, (b) alleged failure to disclose dosage amounts, frequencies and routes of administration for the DNAs, and (c) alleged unpredictability and ineffectiveness of gene therapy (based on a 1995 NIH report).

Without prejudice or disclaimer, applicants have limited the method-of-use claims here to parallel the method-of-use claims allowed in the sibling case 08/486,794 (our Kopchick=1G), which was also before Examiner Saoud. In that application, the

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<sup>2</sup> Human GH is known to bind to the human prolactin receptor. Its affinity for this receptor is comparable to that of human prolactin and human placental lactogen. Therefore, its lactogenic activity may be attributed to activation of the prolactin receptor.

Bovine GH increases milk production in cows. However, it is unlikely that this effect is mediated by the bovine GH receptor alone, as the affinity of bovine GH for this receptor is several orders of magnitude lower than that of prolactin. It is therefore believed that bovine GH activates GH receptors in the mammary glands of cows, and thereby increases milk production.

<sup>3</sup> HGH inhibits insulin, see page 9, lines 18-27. The diabetogenic activity of HGH is believed to be mediated by the GH receptor, but applicants are not bound by this theory.

<sup>4</sup> See Ex. 7 on page 48, and Fig. 11. Note also the reference at page 2, lines 4-6 to bGH increasing the lean-to-fat ratio in cattle.

<sup>5</sup> This activity was discovered by applicants. See page 9, lines 28-30, Ex. 6 at p. 47, and Fig. 10.

Examiner conceded enablement for claims drawn to the use of applicants' polypeptides in "reducing GH activity in a mammal", eschewing any reference to whether such reduction ultimately prevented or treated any disease. Also, the Examiner agreed to allow dependent claims reciting that the polypeptide was administered to a mammal "suffering from" a particular disease.

Claims 46-56 have been amended to parallel, as closely as possible, the corresponding method-of-using-a-polypeptide claims in 08/486,794. Claims 57-60 are dependent on amended claim 46 and do not appear to recite any features which are problematic under the Examiner's view of enablement.

With regard to the issuance of dosage amounts, frequencies, and routes of administration, "gene therapy" is specifically disclosed at page 37, lines 6-12. Pharmaceutical routes of administration are discussed at page 35, lines 2-9. With regard to dosage amounts and frequencies, the person skilled in the art would recognize that the preferred amount of vector administered would be that which would achieve GH levels in the body comparable to that achieved by the polypeptide doses set forth at page 35, lines 31-34. As with a polypeptide, the DNA dose could be determined systematically, see page 35, lines 10-30.

It should be appreciated that since the gene will continue to express the protein, and since the gene will also replicate, the initial dosage is less critical than with conventional pharmaceuticals. It is possible for overall GH antagonist levels to rise for a period of time, whereas, with conventional pharmaceuticals, the amount in the body will never exceed the total amount administered.

The NIH Report is cited to show that gene therapy is "not only extremely unpredictable but is considered ineffective". It appears that this Report is being used to show a lack of

utility/operability. MPEP §2164.07 states that a 112/2 rejection for lack of utility must be made under both 112/1 and 101, should be recited separately from any "pure" 112/1 rejection, and should establish an appropriate basis for imposing a rejection under 101.

In general, a rejection under 101 is proper only if the asserted utility is not believable to a person of ordinary skill in the art. (See MPEP 2107.01 at 2100-38). MPEP §2107.02 states that "as a general rule, if an applicant has initiated human clinical trials for a therapeutic product or process, office personnel should presume that the applicant has established that the subject matter of that trial is reasonably predictive of having the asserted therapeutic utility" (page 2100-42). Thus, the very fact that there are still ongoing human clinical trials of gene therapy is evidence that the therapeutic utility of gene therapy is still considered believable.

Moreover, it is not required that gene therapy be an unalloyed success. MPEP §2107, at page 2100-32, states that "if an invention is only partially successful in achieving a useful result, a rejection of the claimed invention as a whole based on a lack of utility is not appropriate". There is ample evidence that gene therapy can achieve clinical improvements, even if those improvements are not as consistent as clinicians desire.

Grossman, Nature Genetics, 6:335-341 (1994) treated a 29 year old woman homozygous for familial hypercholesterolemia (inherited LDL receptor deficiency) by genetically correcting autologous hepatocytes with a retroviral vector bearing the LDL receptor ex vivo, and then transplanting the cells back into the patient. The patient's LDL/HDL ratio declined from 10-13 before gene therapy to 5-8 following gene therapy, and this improvement was stable for the duration of the treatment (18 months).

Blaese, et al., Science, 270:475-480 (October 20, 1995) reported the initial results on a clinical trial of the effect of retroviral-mediated transfer of the adenosine deaminase (ADA) gene into the T cells of two children with severe combined immunodeficiency syndrome. Conventional enzyme treatment was continued during the trial. Over the course of the trial, the dose of PEGylated ADA enzyme given to each of the patients was decreased by more than half; nonetheless, their immune functions improved. By contrast, worsened immune function has been seen in comparable patients when their dose of enzyme was similarly reduced.

Crystal, Science, 270:404-410 (1995), reviewing the progress of gene therapy, comments

- (a) the evidence that human gene transfer is feasible is overwhelming (from 28 ex vivo and 10 in vivo studies);
- (b) while no human disease has yet been cured by gene transfer, several studies have shown that therapeutic genes can evoke biological responses that are relevant to the gene product and the specific disease state of the recipient (although the quantitative results of the studies have been inconsistent<sup>6</sup>),
- (c) in the case of monogenic hereditary disorders, it is rational to believe that, if the normal gene product could be appropriately expressed at the relevant site, the abnormal biologic phenotype could be corrected,

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<sup>6</sup> Crystal gives, as one example, that "an appropriate biological response to gene transfer...has been observed in some patients in most, but not all, studies of CFTR cDNA transfer". However, as a matter of law, this partial success is sufficient for patentability.

USSN - 08/488,164

and

- (d) adverse events have been rare and have been related mostly to the dose and the manner in which the vectors were administered.

Respectfully submitted,

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Enclosure

- Abstract of the Disclosure
- Copy of the '809 patent
- Fuh, et al. (1992)
- Lowman et al. (1991)
- Fuh and Wells (1995)
- WO97/11178
- Grossman (1994)
- Blaese (1995)
- Crystal (1995)
- Watahiki, et al. (1989)
- Cunningham and Wells (1989)
- Cunningham, et al. (1989)
- Cunningham, et al. (1990)

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